Negative effect of vitamin D on kidney function: a Mendelian randomization study

Mendelian randomization study supports a negative causal effect of vitamin D on kidney function

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Abstract

Background: The kidney plays a central role in the regulation of vitamin D metabolism. It is not clear, however, whether vitamin D influences kidney function. Previous studies have reported conflicting results, which may have been influenced by reverse causation and residual confounding. We conducted a Mendelian randomization (MR) study to obtain unconfounded estimates of the association between genetically instrumented vitamin D metabolites and estimated glomerular filtration rate (eGFR) as well as the urinary albumin-to-creatinine ratio (UACR).

Methods: We performed a two-sample MR study based on three single nucleotide variants associated with 25(OH)D levels: rs2282679, rs10741657, and rs12785878, related to the genes *GC*, *CYP2R1*, and *DHCR7*, respectively. Estimates of the allele-dependent effects on serum 25(OH)D and eGFR/UACR were obtained from summary statistics of published genome-wide association meta-analyses. Additionally, we performed a one-sample MR analysis for both 25(OH)D and $1,25(OH)_2D$ using individual-level data from six cohorts.

Results: The combined MR estimate supported a negative causal effect of log-transformed 25(OH)D on log-transformed eGFR (beta −0.013, p = 0.003). The analysis of individual-level data confirmed the main findings, and also revealed a significant association of $1,25(OH)_2D$ on eGFR (beta = −0.094, p = 0.008). These results show that a 10% increase in serum 25(OH)D levels cause a 0.3% decrease in eGFR. There was no effect of 25(OH)D on UACR (beta 0.032, p = 0.265).

Conclusion: Our study suggests that circulating vitamin D metabolite levels are negatively associated with eGFR. Further studies are needed to elucidate the underlying mechanisms.

Keywords: albuminuria, glomerular filtration rate, causality, Mendelian randomization, vitamin D

Introduction

The kidneys play a central role in the regulation of vitamin D metabolism. They produce the 25-hydroxyvitamin D3-1α-hydroxylase enzyme that converts 25-hydroxyvitamin D3 (25[OH]D) to the active form 1α,25-dihydroxyvitamin D3 ($1,25[OH]_2D$). As a consequence, during progression of chronic kidney disease (CKD), circulating levels of $1,25(OH)_2D$ decline (1). Conversely, whether vitamin D status might affect kidney function, is less clear. Because of the aforementioned interrelation between renal function and vitamin D metabolism, cross-sectional designs are not appropriate to address this question, because they do not allow for reconstructing a temporal relationship between changes of vitamin D status and subsequent kidney function alterations. To date, several observational studies investigated the effects of vitamin D status on renal function with inconsistent results, with some suggesting a beneficial effect (2,3) while others reporting no association or mixed results(4–6), and even others suggesting a harmful effect(7). In all these studies, decline of kidney function was assessed from baseline to follow-up, whereas vitamin D status was measured at baseline only. As no information on vitamin D metabolites was available over time, the possibility that the findings might reflect reverse causation cannot be ruled out. Furthermore, due to the observational nature of these studies, the associations between vitamin D and renal function might be influenced by confounding e.g. from inflammation, diabetes mellitus, hypertension, or body mass index(8). The Mendelian randomization (MR) approach analyzes the relationship between exposure and outcome by an unconfounded genetic instrument. It might therefore be useful to overcome the limitations of observational studies(9,10) and thus help to disentangle the relationship between vitamin 25(OH)D and markers of kidney function. In the present study, we investigated the relationship between the levels of circulating 25(OH)D and two measures of kidney function, namely the estimated

glomerular filtration rate (eGFR) and the urinary albumin to creatinine ratio (UACR), with a MR approach using data from published literature. To further explore the association, we additionally analyzed individual-level data from six population-based studies and extended the MR analyses to assess a causal effect of $1,25(OH)_2D$ levels on eGFR. Because no genome-wide association study (GWAS) on $1,25(OH)_2D$ levels was available, we generated and analyzed individual level data to close this gap and carry out a MR analysis for $1,25(OH)_2D$.

Materials and Methods

Phenotypes and instrumental variables

In the initial two-sample MR, we included published genetic association summary statistics data on 25(OH)D(11), serum creatinine based eGFR(12), and urinary albumin-to-creatinine ratio (UACR)(13) based on n=33,868, 133,720, and 54,448 individuals, respectively. The one-sample two-stage least-squares MR of 25(OH)D and vitamin $1,25(OH)_2D$ on eGFR was calculated using data of 16,442 subjects from six studies (COLAUS, LURIC, ORCADES, PREVEND, SHIP and SHIP-Trend) and 5,123 subjects from two studies (LURIC and PREVEND), respectively. None of the studies was included in the published 25(OH)D GWAS meta-analysis (Table 1, Supplemental Material). Additional 2,696 samples of the PREVEND study without available genetic information but with measured levels of 25(OH)D and vitamin $1,25(OH)_2D$ were included in the non-instrumented stratified analyses. All individuals analyzed in this project were of European ancestry.

As an initial selection of the instruments, we considered single nucleotide polymorphisms (SNPs) strongly associated with 25(OH)D levels as reported by Wang *et al.*(11) and Ahn *et al.*(14). Given the different covariate adjustments and the smaller sample size of Ahn *et al.* compared to Wang *et al.*, we focused on the association results of the latter study only: the

exonic SNP rs4588 and rs2282679 in *GC*, rs12785878 in *NADSYN1*, rs10741657 upstream *CYP2R1*, and rs6013897 downstream *CYP24A1*. The beta values from the published GWAS meta-analyses on eGFR(12) and UACR(13) were used as effect estimates of the SNPs on kidney function. As the SNP rs4588, which was also in strong linkage disequilibrium (LD) with rs2282679, was not included in these two meta-analyses, it was excluded from subsequent analyses.

All loci significantly associated with 25(OH)D in the study of Wang *et al.* were evaluated whether they represented valid instruments for MR studies (15), taking into account both biological and statistical criteria. That is, SNPs were evaluated for a strong association with 25(OH)D and for potential pleiotropy (association with confounders of the association of vitamin D with kidney function to ensure that the SNPs were only associated with kidney function through 25(OH)D)(16). Briefly, the SNP rs2282679 is located in an intron of *GC* which encodes a vitamin D binding protein that transports vitamin D metabolites in the blood. The intronic SNP rs12785878 of *NADSYN1* has several SNPs in high LD located in or near the 7-dehydrocholesterol reductase encoding gene *DHCR7*. This product of this gene as well as the cytochrome P450 family 2 subfamily R member 1 encoded by *CYP2R1* is involved in vitamin D synthesis. However, we observed a significant association of rs6013897 with eGFR ($p = 7.5x10^{-10}$). This SNP is located in close vicinity of *CYP24A1* which encodes an enzyme deactivating vitamin $1,25(OH)_2D$(17,18) in the kidney as well as other tissues, and thus we cannot exclude that the SNP affects kidney function *via* other mechanisms than exclusively through 25(OH)D levels. Therefore, this SNP could not be considered as a valid instrument for the analysis of kidney function as an outcome and was excluded from the MR analyses. The F-statistics of the remaining three SNPs rs2282679, rs10741657 and rs12785878 were higher than the recommended value of ten(10) according to the ~6000

individuals included in Berry *et al.* supporting these SNPs as strong instruments for both our two- and one-sample MR studies (Figures 1 and 2).

Because in Wang *et al.* no effect estimates were available because the GWAS were pooled using a z-score based meta-analysis, effect estimates required for the MR as well as their standard errors were estimated using the provided allele frequency, effect direction, p-value, and sample size as described previously(19). The phenotypic variance was set to one whereas the effect estimates represent the change of one standard deviation unit of the log-transformed and covariate-adjusted 25(OH)D values. We used the 3,159 SHIP-1 samples with available SNP information and 25(OH)D values to test the method and compare the estimated effect sizes with the calculated ones (Supplementary Table 1).

Statistical analyses

For the analyses, all traits were transformed using the natural logarithm. The effect sizes of the 25(OH)D traits correspond to one standard deviation unit of the log-transformed values adjusted for sex, age and body mass index, as well as season (log[25(OH)D])(11). The effect sizes of the kidney function traits correspond to a unit change of log-transformed eGFR (4-parameter MDRD equation using calibrated creatinine measurements, log[eGFR]) and UACR values, respectively(12,13).

The causal effect estimates of serum 25(OH)D on kidney function, based on the published GWAS meta-analysis results, were calculated using an inverse-variance meta-analysis of the two-sample MR triangulation per SNP (20), with its estimated effect sizes on 25(OH)D, and the effect sizes and their standard errors of the corresponding kidney trait as input.

MR based on individual study data of 25(OH)D and vitamin $1,25(OH)_2D$ on eGFR was calculated using a one-sample two-stage least-squares analysis. The standardized residuals

of the log-transformed vitamin D traits adjusted for the same covariates as in the GWAS of Wang *et al.* (i.e. sex, age, body mass index, season) were used as exposure (log[25(OH)D] and log[1,25$(OH)_2$D], respectively). The log(eGFR) was used as outcome. In each study, linearity of the relationship between log(25(OH)D) and log(eGFR) was assessed via locally-weighted polynomial regression, as implemented in the R-package *lowess*. The standard deviation of log(25[OH]D) that was needed to transform the effect estimates of the standardized residuals back to the original log-scale for calculating the relative change of the causal effects was estimated in the SHIP cohort (σ=0.44).

Simple (non-instrumented) association analyses were performed by fitting linear regression models of log(eGFR) values on log(25(OH)D) (n=19,138) and log(1,25$[OH]_2$D) (n=7,819), respectively, using the same cohorts and adjustments for covariates included in the one-sample MR (Table 1, Supplemental Material). Stratified analyses were performed according to the chronic kidney disease (CKD) status, defined as eGFR <60 ml/min/1.73m².

All analyses were performed using the R software package(21). Individual data analyses were performed in each cohort separately by distributing a centralized script and meta-analyzing the results afterwards by inverse-variance weighting. Significance of the statistical tests was set at α= 0.025, corresponding to a level of 0.05 divided by the two outcomes tested (eGFR and UACR).

Results

The two-sample MR analysis for kidney function supported a negative causal effect of 25(OH)D levels on eGFR (beta = −0.013, p = 0.003, Figure 1, Supplementary Figure 1A) with relatively low heterogeneity between the three instruments (I^2=28%). This result was reinforced by the one-sample MR analysis: beta = −0.033, p = 0.013, Supplementary Figure

2A, Supplementary Table 2). Additionally, a one-sample MR showed a significant association of vitamin 1,25$(OH)_2$D on eGFR (beta = −0.094, p = 0.008, Supplementary Figure 2B, Supplementary Table 2). No indication of a non-linear relation between these traits was observed (Supplementary Figures 4 and 5). There was no evidence of a causal association between Vitamin D and UACR (beta = 0.032, p = 0.265, I^2=0%, Figure 2, Supplementary Figure 1B) using the association results of published GWAS.

To investigate possible reasons for the previously reported, conflicting association between lower levels of 25(OH)D and impaired kidney function(2,5), we performed a non-instrumented association between 25(OH)D levels and eGFR stratified by CKD status. We observed an negative association between eGFR and 25(OH)D in the 18,029 non-CKD individuals (beta = −0.025, p = $1.1x10^{-66}$, Supplementary Figure 3E) and a positive association in the 1,109 CKD individuals (beta = 0.014, p = $3.1x10^{-4}$, Supplementary Figure 3C). In contrast, vitamin 1,25$(OH)_2$D was positively associated with eGFR in both the 448 CKD subjects (beta = 0.044, p = $5.2x10^{-13}$, Supplementary Figure 3D) and in the 7,371 non-CKD individuals (beta = 0.006, p = 0.002, Supplementary Figure 3F). Comparing the causal one-sample MR effects with the observed (non-instrumented) association results of eGFR using the same individuals, the effect directions were concordant for 25(OH)D (beta = −0.022, p = $7.2x10^{-33}$, Supplementary Figure 3A) but not for vitamin 1,25$(OH)_2$D (beta = 0.037, p = $3.0x10^{-33}$, Supplementary Figure 3B).

Based on the estimated causal effects, a 10% increase in serum 25(OH)D levels corresponds to a 0.3% decrease in eGFR (two-sample MR). The estimated change in 25(OH)D levels depending on the number of trait increasing alleles per instrument varies from 3% to 18% (Figure 3), whereas individuals being homozygous for all trait increasing alleles of these three

SNPs are estimated to have 37% higher 25(OH)D levels compared to individuals without trait increasing alleles.

Discussion

Using the largest available GWAS on vitamin D metabolites and eGFR, we observed a potentially negative causal relationship between serum 25(OH)D and kidney function defined by eGFR. This association could be reinforced by individual study MR analyses. Additionally, a similar negative causal association was revealed for vitamin 1,25$(OH)_2$D with eGFR.

Although these results are consistent with the observational association results of 25(OH)D and kidney function in the population-based cohorts participating in this study, they contradict the previously reported associations of lower vitamin D status with increased risk of CKD progression(2,5) assessed as either rapid decline in eGFR or risk of incident end-stage renal disease. However, when limiting our observational analyses to individuals with CKD, the effect directions were concordant with those reported for CKD progression from previous observational studies. A potential explanation for this observation could be an impaired re-absorption of vitamin D metabolites in the proximal tubules due to a substantially reduced kidney function, resulting in reduced serum vitamin D levels. This reverse causation could mask a possible causal effect of higher vitamin D levels on lower eGFR. The outcomes analyzed in these two previous studies suggest that individuals with low kidney function at baseline are more likely to be included in the group of CKD progressors than the individuals with normal kidney function, which in turn could lead to confounding of the observed association of vitamin D status with kidney function. Supporting our explanation, other population-based longitudinal studies, which either excluded patients

with CKD at baseline or did not use a rapid eGFR decline for case definition did not show a significant positive association of vitamin D status with change in kidney function(3,4,6,7).

Even though associations of vitamin D status with change in albuminuria were reported previously(3,6,22), no causal effect was shown in our study using two-sample MR analysis. Besides the possibility that no true causal effect exists, our MR analyses included only cross-sectional measurements of UACR to assess albuminuria, it was limited in detecting non-linear log-log transformed causal associations of 25(OH)D on UACR, and may still have been underpowered. Another issue might be the biological variability of UACR as well as the lower precision of UACR assessment in healthy individuals compared with eGFR because of limit-of-detection issues with the urinary albumin assay. The latter issue could affect the effect magnitude of genetic association estimates(23).

As both exposure and outcome were log-transformed prior to the analysis, a non-linear causal relation between untransformed 25(OH)D and eGFR is likely. However, the causal effect with respect to the relative change on the original scale of the traits is rather small, suggesting that other factors not directly related to 25(OH)D levels are clinically more important for kidney function.

Several limitations apply to our study affecting also the significant causal associations of vitamin D traits on eGFR. Because LURIC is a hospital-based cohort selected for an acute coronary syndrome, a one-time estimate of glomerular filtration rate may not accurately represent CKD status. However, the LURIC study was included in previous genetic analyses that gave rise to clear and homogeneous signals. The cross-sectional data limits possible interpretations of the molecular mechanisms underlying the causality. In particular, it would be of interest whether an increase in 25(OH)D levels causes a decline in eGFR or reduced 25(OH)D levels have a protective effect on kidney function.

There are different GFR estimating methods available, including the CKD-EPI equation, which may provide more precise estimates in population-based samples compared to the MDRD formula(24). However, no large-scale GWAS based on CKD-EPI eGFR was conducted until today. For this reason, we used the results of the GWAS of logarithm-transformed cystatin-C-based eGFR, carried out on 33,152 participants by the CKDGen Consortium(12) to assess the robustness of our causal estimates. The causal effect of 25(OH)D on the cystatin-based eGFR was of -0.014 ($p = 0.11$), thus almost identical to that obtained with the MDRD-based estimate. The lack of statistical significance is expected given the substantially smaller sample size.

Furthermore, there is no readily available explanation for our finding of a negative association between 25(OH)D and eGFR. Circulating levels of 25(OH)D are generally used to gauge vitamin D status as they increase dose-dependently following vitamin D administration. However, the formation of $1,25(OH)_2D$ is tightly regulated and unlikely to increase due to higher levels of 25(OH)D, as shown by randomized controlled trials in which supplementation with vitamin D did not consistently cause an increase in $1,25(OH)_2D$ levels(25,26). That circulating levels of 25(OH)D might fail to capture the complexity of the relationship with kidney function is confirmed by the study by Rebholz *et al.*(5), in which the authors found no association between 25(OH)D and risk of end-stage renal disease, but a significant association for vitamin D binding protein.

Vitamin D is a lipid soluble hormone, whereas only free vitamin D is passing the cell membrane (with the exception of megalin mediated vitamin D uptake in the proximal tubule of the kidney and the parathyroid gland) and interacts with the vitamin D receptor(27,28). Therefore, additional studies using measurements of free vitamin D could help to shed light on the biological mechanisms explaining the causal association revealed in our study.

Finally, all analyses were performed based on data of European ancestry individuals thus the generalization of the findings with respect to other ethnicities needs to be evaluated.

In conclusion, our results demonstrate that levels of both 25(OH)D and $1,25(OH)_2D$ are negatively associated with eGFR. Further studies are warranted to confirm this finding and to look at potential mechanisms for this association.

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Conflict of Interest Statement

W.M. is employed with Synlab Holding Deutschland GmbH. The sponsor had no role in the study design, analyses, drafting of the manuscript, or the decision to publish.

Authors' Contributions

Statistical Methods and Analysis: A.T., C.L.B., M.E.K., M.H.d.B., P.K.J., P.v.d.H. Subject Recruitment: I.G., J.F.W., M.B., M.N., P.V., T.C., W.M. Study Management: J.F.W., M.B., M.N., P.V., W.M. Study Design: A.K., A.T., C.P., G.G., P.M.F., W.M. Genotyping: G.H., J.F.W., M.E.K.,

T.C. Drafting of manuscript: A.T., P.M.F. Interpretation of Results: A.K., A.T., C.P., G.G., K.E., M.E.K., P.M.F., S.P. Critical review of manuscript: all authors.

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Tables

Table 1: Characteristics of cohorts included in the one-sample MR and non-instrumented analyses

Study name	Study design	Imputation panel and software	Genotyping platform	Vitamin D measurement	Serum creatinine measurement	Sample size	Women %	Age in years (SD)	eGFR in ml/min/ 1.73 m² (SD)	Vitamin D in µg/l (SD)	BMI in kg/m² (SD)
COLAUS	population based	HRC (IMPUTEv2)	Affymetrix 500k	LC/MS/MS and LC/HRMS (Bruce SJ *et al.* 2013)	Jaffe kinetic compensated method	5406	53.0	53.4 (10.8)	89.9 (19.5)	48.5 (22.7)	25.8 (4.5)
LURIC	hospital based	1000Gv3 (IMPUTEv2)	Affymetrix SNP 6.0	^{125}I RIA, Vitamin D, DiaSorin	Jaffe, 1997-2000	3025	29.6	62.9 (10.4)	80.2 (19.7)	17.3 (9.8)	27.5 (4.0)
ORCADES	population based	1000Gv3 (IMPUTEv2)	Illumina Hap300, Omni1, OmniExpress	liquid chromatography -tandem mass spectrometry	Jaffe	1774	59.2	54.0 (14.5)	88.1 (23.7)	35.1 (18.7)	27.9 (5.0)
PREVEND	population based	1000Gv3 (IMPUTEv2)	Illumina HumanCytoSNP-12 BeadChip	LC-MS/MS	Roche modular	2098	48.4	51.0 (13.0)	96.1 (17.1)	23.5 (9.2)	26.1 (4.3)
SHIP	population based	1000Gv3 (IMPUTEv2)	Affymetrix SNP 6.0	IDS-iSYS 25-Hydroxy Vitamin D assay	Jaffe, 2002	3158	51.8	54.5 (15.2)	90.3 (23.4)	19.4 (9.5)	28.0 (4.9)
SHIP-Trend	population based	1000Gv3 (IMPUTEv2)	Illumina Omni 2.5	IDS-iSYS 25-Hydroxy Vitamin D assay	Jaffe, 2008	981	56.2	50.1 (13.7)	92.4 (22.1)	25.8 (11.2)	27.3 (4.6)

Legends to Figures

Figure 1: Association results of MR analyses with eGFR

The figure shows the GWAS meta-analysis association results of the instruments (SNPs) with log(25[OH]D) and log(eGFR) (solid arrows) used to estimate the causal effect (dashed arrow) between 25(OH)D and eGFR through Mendelian randomization (MR). a. , b., c. represent the association results of the respective instruments.

Figure 2: Association results of MR analyses with UACR

The figure shows the GWAS meta-analysis association results of the instruments (SNPs) with log(25[OH]D) and log(UACR) (solid arrows) used to estimate the causal effect (dashed arrow) between 25(OH)D and UACR through Mendelian randomization (MR). a. , b., c. represent the association results of the respective instruments.

Figure 3: Change in 25(OH)D depending on the instruments

The figure illustrates the per cent change of 25(OH)D levels for each SNP used as an instrument depending on the number of trait increasing alleles. The three instruments are rs10741657 upstream *CYP2R1*, rs12785878 in *NADSYN1*, and rs2282679 in *GC*.

Figure 1

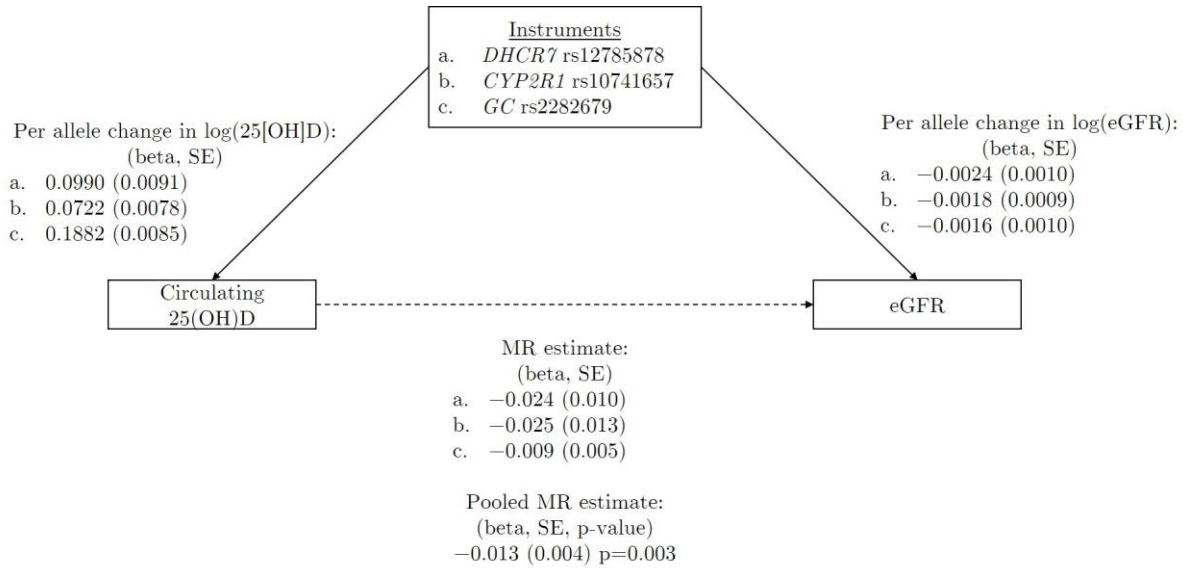


Figure 2

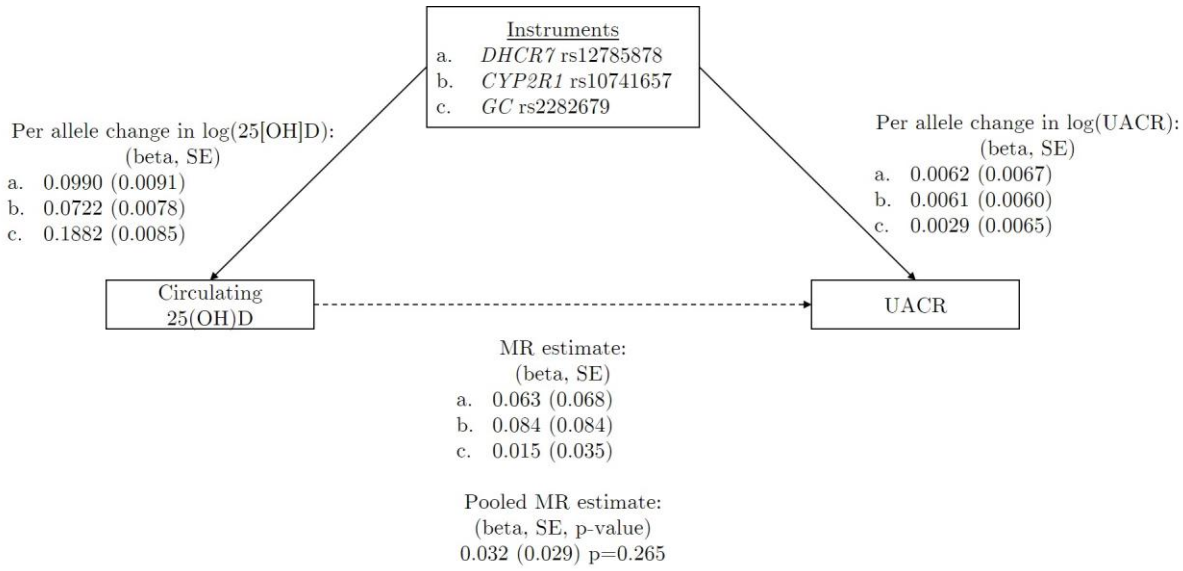
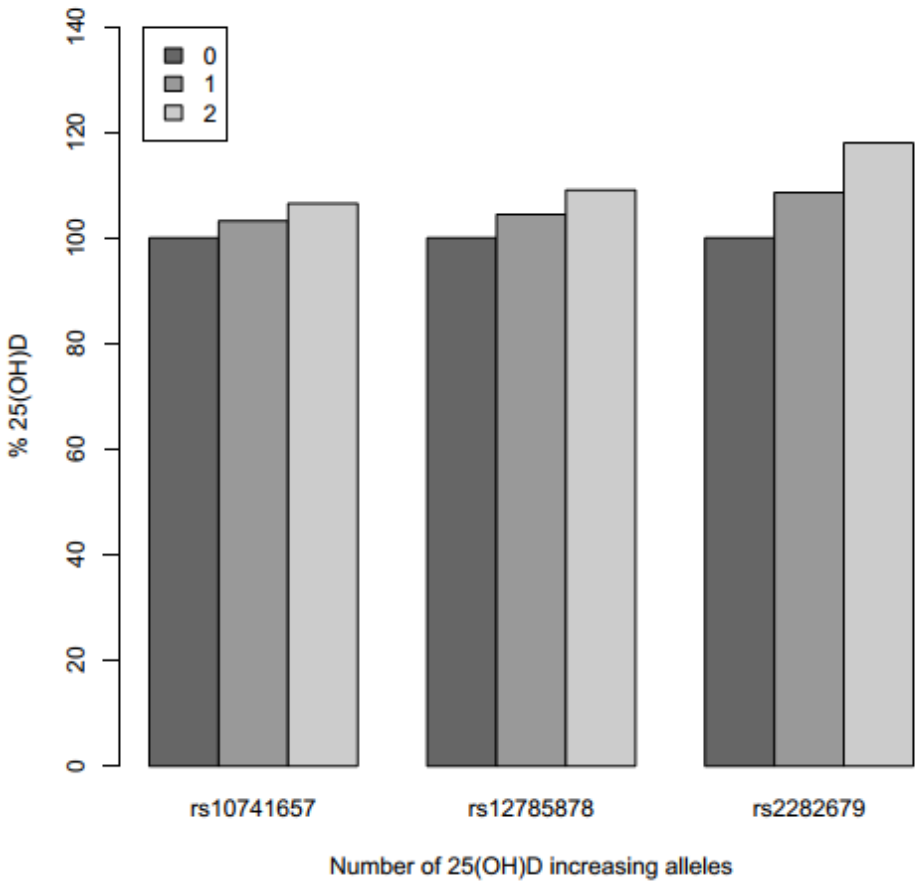


Figure 3


0
1
2
% 25(OH)D
0
20
40
60
80
100
120
140
rs10741657
rs12785878
rs2282679
Number of 25(OH)D increasing alleles